Institute Report No. 411

Dermal Sensitization Potential of DIGL-RP Solid Propellant in Guinea Pigs

Yvonne C. LeTellier, BS
Larry D. Brown, DVM, LTC, VC
and
Don W. Korte, Jr., PhD, LTC, MSC

MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

October 1989

Toxicology Series: 179

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Dermal Sensitization Potential of DIGL-RP Solid Propellant in Guinea Pigs (Toxicology Series 179)--LeTellier *et al.*

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

This research was conducted in compliance with the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23, as prepared by the Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

Donald G Corby 30 Oct 87

Donald G. Corby (date)
COL, MC
Commanding

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION / AVAILABILITY OF REPORT APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No.: 411	5 MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Mammalian Toxicology Division of Toxicology 6b. OFFICE SYMBOL (If applicable) SGRD-ULE-T	7a. NAME OF MONITORING ORGANIZATION US Army Biomedical Research and Development Laboratory
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5010
8a. NAME OF FUNDING / SPONSORING ORGANIZATION US Army Medical Research & Development Command 8b OFFICE SYMBOL (If applicable)	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 62720 PROJECT NO: A835 TASK NO: AB WORK UNIT ACCESSION NO: DA303913

11. TITLE (Include Security Classification)
(U) Dermal Sensitization Potential of DIGL-RP Solid Propellant in Guinea Pigs

12. PERSONAL AUTHOR(S)
YC LeTellier, LD Brown, and DW Korte, Jr.

13a. TYPE OF REPORT	13b. TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Institute	FROM 4APR86 TO 16MAY86	October 1989	34

16. SUPPLEMENTARY NOTATION
Toxicology Series No. 179

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	DIGL-RP Solid Propellant, Diethyleneglycol Dinitrate, Mammalian Toxicology, Munition, Dermal Sensitization, Buehler Test, Guinea Pig

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

DIGL-RP Solid Propellant was evaluated for its potential to produce dermal sensitization in male guinea pigs. The Buehler test, which utilizes repeated closed patch inductions with the test compound, was used for this evaluation. No evidence that DIGL-RP Solid Propellant induced sensitization was obtained in the study.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	21 ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL DONALD G. CORBY, COL, MC	22b TELEPHONE (Include Area Code) (415) 561-3600 22c OFFICE SYMBOL SGRD-ULE

DD Form 1473, JUN 86 Previous editions are obsolete

ABSTRACT

Cont'd From Pg. 1

DIGL-RP Solid Propellant was evaluated for its potential to produce dermal sensitization in male guinea pigs. The Buehler test, which utilizes repeated closed patch inductions with the test compound, was used for this evaluation. No evidence that DIGL-RP Solid Propellant induced sensitization was obtained in the study. Keywords:

Key Words: Dermal Sensitization, Mammalian Toxicology, DIGL RP Solid Propellant, Diethyleneglycol Dinitrate, Buehler Test, Guinea Pigs, Munition. (AU)

PREFACE

TYPE REPORT: Dermal Sensitization GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NO.: 85026

STUDY DIRECTOR: Don W. Korte, Jr., PhD, LTC, MSC
Diplomate, American Board of Toxicology

PRINCIPAL INVESTIGATOR: Yvonne C. LeTellier, BS

CO-INVESTIGATOR: Larry D. Brown, DVM, LTC, VC, Diplomate,
American College of Veterinary Preventive Medicine,
American Board of Toxicology.

PATHOLOGIST: Michael V. Slayter, DVM, MAJ, VC

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: DIGL-RP Solid Propellant

INCLUSIVE STUDY DATES: 4 April - 16 May 1986

OBJECTIVE:

The objective of the study was to evaluate the dermal sensitization potential of DIGL-RP Solid Propellant in guinea pigs.

ACKNOWLEDGMENTS

SP4 Gregory A. Rothhammer, SP4 Scott Schwebe, SP4 Theresa L. Polk, Obie Goodrich, and Richard Spieler provided technical assistance, animal care, and facilities management.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 85026 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr. 30 Oct 89

DON W. KORTE, JR., PhD / DATE
LTC, MSC
Study Director

Yvonne C. LeTellier 30 Oct 89

YVONNE C. LETELLIER, BS / DATE
DAC
Principal Investigator

Larry D. Brown 13 July 1989

LARRY D. BROWN, DVM / DATE
LTC, VC
Co-Investigator

Conrad Wheeler 30 Oct 89

CONRAD R. WHEELER, PhD / DATE
DAC
Analytical Chemist

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH

PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

SGRD-ULZ-QA

26 October 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85026

1. This is to certify that the protocol for LAIR GLP Study 85026 was reviewed on 10 May 1985.

2. The institute report entitled "Dermal Sensitization Potential of DIGL-RP Solid Propellant in Guinea Pigs," Toxicology Series 179, was audited on 25 October 1989.

Carolyn M. Lewis

CAROLYN M. LEWIS
Diplomate, American Board of Toxicology
Quality Assurance Auditor

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

Dermal Sensitization Potential of DIGL-RP Solid Propellant in Guinea Pigs– LeTellier *et al.*

INTRODUCTION

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in new propellant formulations. However, considerable gaps in the toxicology data of the compounds were identified during a review of their health effects (1) conducted for the US Army Biomedical Research and Development Laboratory (USABRDL). Consequently, USABRDL has tasked the Division of Toxicology, Letterman Army Institute of Research (LAIR), to conduct an initial health effects evaluation of the proposed replacement nitrate esters. This initial evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP, includes the Ames mutagenicity assay, acute oral toxicity studies in rats and mice, acute dermal toxicity study in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

Objective of Study

The objective of this study was to determine the dermal sensitization potential of DIGL-RP Solid Propellant in guinea pigs.

MATERIALS

Test Substance

Chemical Name: DIGL-RP Solid Propellant

LAIR Code Number: TP57

Description: Solid black cylinders (stick configuration)

Lot Number: RAD83M001S169

DIGL-RP Solid Propellant was received in the stick configuration. It was ground into a fine powder for this study (Appendix A). Other test substance information is presented in Appendix A.

Vehicle

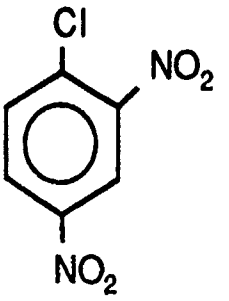
Isotonic saline (Viaflex®, Sodium Chloride Injection, USP; Travenol Laboratories, Inc., Deerfield, IL) was used as the vehicle for the test compound and as a component of the postive control vehicle.

Positive Control

Chemical Name: Dinitrochlorobenzene (DNCB)

Chemical Abstracts Service Registry No.: 97-00-7

Chemical Structure:



Molecular Formula: $C_6H_3N_2O_4Cl$

Other positive control substance information is presented in Appendix A.

Vehicle for Positive Control

A 0.1% solution of DNCB was prepared on 14, 21, and 28 April and 12 May 1986. The vehicle for DNCB was a propylene glycol (3%) and isotonic saline (97%) mixture. Propylene glycol (lot number 36485) was obtained from Dow Chemical Company (Freeport, TX).

Animal Data

Sixty male albinc guinea pigs, Hartley strain (Charles River Breeding Laboratories, Kingston, NY), from a shipment received on 4 April 1986 were assigned to this study. They were identified individually with ear tags. Two animals (86E0130, 86E0147) were selected for quality control necropsy evaluation on receipt. Animal weights on the day of receipt ranged from 178 to 235 g. Additional animal data appear in Appendix B.

Husbandry

Guinea pigs assigned to this study were caged individually in stainless steel, wire mesh cages in racks equipped with automatically flushing dump tanks. The diet, fed *ad libitum*, consisted of Certified Purina Guinea Pig Chow® Diet 5026 (Ralston Purina Company, Checkerboard Square, St. Louis, MO); water was provided by continuous drip from a central line. Temperature within the animal room was maintained in the range from 21.6° to 25.6°C. Relative humidity was maintained in the range of 40% to 59% with spikes to 72% associated with room cleaning. The photoperiod was 12 hours of light per day.

METHODS

This study was conducted in accordance with LAIR SOP-OP-STX 82 "Buehler Dermal Sensitization Test" (2) and EPA guidelines (3).

Group Assignment/Acclimation

The guinea pigs were quarantined for 12 days before administration of the first induction dose. During the quarantine period, they were checked daily for signs of illness and weighed once a week. Fifteen animals were assigned to each of four groups by a stratified randomization technique based on their body weights.

Dose Levels

Dermal sensitization potential was evaluated in a test group receiving three weekly induction doses of 100% (w/v) DIGL-RP and, after a two-week delay, a challenge dose at the same concentration. Dinitrochlorobenzene, a known potent sensitizing agent (4), was used as the positive control. It was applied to another group, at a 0.1% concentration, in the same dosing sequence as the test compound. The vehicle control group received isotonic saline only for the three weekly induction doses and the challenge dose. The negative control group received 100% (w/v) DIGL-RP only on the day of challenge dosing.

Compound Preparation

DIGL-RP was received in the stick configuration and ground in a Spex Industries freezer mill to a fine powder. The ground DIGL-RP was sieved through a 80-mesh screen before mixing with saline to form a 100% (w/v) concentration for testing. A 0.5 g dose of this concentration of DIGL-RP was shown to be non-irritating in pilot studies. The dinitrochlorobenzene (DNCB) dosing solution was prepared by first adding 30 mg DNCB to 1.0 ml of propylene glycol and heating until it dissolved (approximately 40°C). To this, 29 ml of 0.9% sodium chloride solution were added, to give a final concentration of 0.1% (w/v). This solution was heated to 65°C and vortexed before application to keep the DNCB in solution. DNCB solutions were prepared fresh for each application day.

Test Procedures

The closed patch dermal sensitization test procedures utilized in this study were developed by Buehler and Griffith (5-7) to mimic the repeated-insult patch test for humans. Test compounds were applied for six hours under a closed patch once a week for three weeks during the induction phase. The same application site was used for each induction dose. To distinguish between reactions from repeated insult and sensitization, duplicate patches of the challenge dose were applied, one on the old site and one on a new site.

To distinguish between reactions from primary irritation and sensitization, a negative control group was added which received only the challenge dose.

During the induction phase, the test and positive control groups were dosed with 0.5 ml of 100% (w/v) DIGL-RP applied topically under a 2.5-cm^2 gauze patch. This procedure was performed for three consecutive weeks (15, 22, and 29 Apr 86). Twenty-four hours before each dosing, a 7.6-cm^2 area on the left flank of the animal was clipped with electric clippers (Oster® Model A5, size 40 blade, Sunbeam Corp., Milwaukee, WI) and then shaved with an electric razor (Norelco® Speed Razor Model HP1134/S, North American Phillips Corp., Stamford, CT). The patch was taped with Blenderm® hypoallergenic surgical tape (3M Corp., St. Paul, MN) to the same site each time, and the animal was wrapped several times with Vet Wrap® (3M Corp., St. Paul, MN). The patch was left in place for six hours. When the wrap and patch were removed, the area under the patch was gently wiped of any excess compound using a saline-moistened gauze and the site was marked for scoring.

Animals were challenged two weeks (13 May 86) following the third induction dose. Test group and positive control group animals received two 0.5-ml doses each of DIGL-RP or DNCB, respectively, one applied to the old site on the left flank and the other to a new site on the right flank. Negative control animals received only a single 0.5-ml dose of DIGL-RP, applied to the left flank. Vehicle control animals received only a single 0.5-ml dose of saline, applied to the left flank. Procedures for clipping, shaving, and wrapping and the exposure period remained the same.

In Buehler's procedure, skin reactions are scored 24 and 48 hours after the challenge dose only. In the present study, skin reactions were scored 24, 48, and 72 hours after each induction dose as well as 24, 48, and 72 hours after the challenge dose. Skin reactions were assigned scores according to Buehler's grading system: 0 (no reaction), 1 (slight erythema), 2 (moderate erythema), and 3 (marked erythema). Results are expressed in terms of both incidence (the number of animals showing responses of 1 or greater at either 24, 48, or 72 hours) and severity (the sum of the test scores

divided by the number of animals tested). Results from the left flank are compared with right flank and with the negative control group.

Some modifications of Buehler's procedures were made. Instead of placing animals in restraint during the 6-hour exposure period, the animals were wrapped several times with an elasticized tape to hold the patch in place. Consequently, the animals were able to move about freely in their cages during the exposure period. Buehler and Griffith (7) also recommended depilating the day before the challenge dose. For consistency with induction procedures, this step was replaced by clipping the animals.

The animals were observed daily for clinical signs and weight gain was monitored during the study. At the conclusion of the study, a necropsy was performed on each animal. A historical listing of study events appears in Appendix C.

Changes/Deviations

This study was conducted in accordance with the protocol and applicable amendments with two exceptions. Animal 86E0183 of the negative control group had its wrappings in place 24 hrs instead of 6 hrs as scheduled for the challenge dose. Animal 86E0152 of the DIGL-RP treatment group was not shaved the night before the challenge dose and therefore was removed from the study. It is believed that these deviations had no effect on the outcome of this study.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Experimental

Table 1 summarizes the incidence of reactions 24, 48, and 72 hours after each dose. Slight erythema was observed in two animals after the first induction dose of DIGL-RP and in two other animals after the second induction dose of DIGL-RP. No other reactions were observed in the animals in the DIGL-RP test group. This relative lack of response is reflected in Table 2 which depicts the severity of skin reactions. Response severity for each group is calculated by summing the scores of responding animals and dividing by the total number of animals within that group. For DIGL-RP, the maximum severity scores were 0.13 and 0.07, obtained after the first and second induction doses, respectively.

Positive Control

Dinitrochlorobenzene produced a marked response at all time points (Table 1). All DNCB-treated animals exhibited a response 24 hours following the second or third induction and challenge doses. These reactions persisted, yielding scorable effects in all the animals at 48 hours and at 72 hours after dosing. Severity scores for these responses to DNCB ranged from 0.53 to 1.6 at the 24-hour scoring period (Table 2). By 48 hours the reactions had peaked, ranging from 0.53 to 1.87. At 72 hours the reactions had subsided to the same score as for the 24-hour observation.

Negative and Vehicle Controls

No response was observed in the negative control (challenge dose of DIGL-RP) group or the vehicle control (isotonic saline) group. Individual 24 hour, 48-hour, and 72-hour dermal scores for all animals appear, by group, in Appendix D.

Clinical Signs

All animals were healthy and gained weight during the study. Individual body weight data are presented in Appendix E.

TABLE 1: Incidences of Skin Reactions

Test Group	Induction			Challenge	
	First	Second	Third	Right	Left
			24 Hours		
DNCB	8/15	15/15	15/15	14/15	15/15
Vehicle Control	0/15	0/15	0/15	---	0/15
DIGL RP	0/15	1/15	0/15	0/14	0/14
Negative Control	---	---	---	---	0/14
			48 Hours		
DNCB	8/15	15/15	15/15	14/15	15/15
Vehicle Control	0/15	0/15	0/15	---	0/15
DIGL RP	0/15	1/15	0/15	0/14	0/14
Negative Control		---	---	---	0/15
			72 Hours		
DNCB	8/15	15/15	15/15	14/15	15/15
Vehicle Control	0/15	0/15	0/15	---	0/15
DIGL-RP	2/15	0/15	0/15	0/14	0/14
Negative Control	---	---	---	---	0/15

TABLE 2: Severity of Skin Reactions

Test Group	Induction			Challenge	
	First	Second	Third	Right	Left
		24 Hours			
DNCB	0.53	1.47	1.47	1.33	1.6
Vehicle Control	0.0	0.0	0.0	--	0.0
DIGL-RP	0.0	0.07	0.0	0.0	0.0
Negative Control	---	---	---		0.0
		48 Hours			
DNCB	0.53	1.47	1.2	1.47	1.87
Vehicle Control	0.0	0.0	0.0		0.0
DIGL-RP	0.0	0.07	0.0	0.0	0.0
Negative Control	---	---	---	---	0.0
		72 Hours			
DNCB	0.53	1.53	1.4	1.33	1.6
Vehicle Control	0.0	0.0	0.0		0.0
DIGL-RP	0.13	0.0	0.0	0.0	0.0
Negative Control	---	---	---		0.0

Pathology Findings

A necropsy was performed on all study animals. Lobular liver necrosis was identified in at leat half of the study animals. This is a common finding of unknown etiology in otherwise healthy guinea pigs. The complete pathology report is presented in Appendix F.

DISCUSSION

Dermal Irritation and Sensitization

Most skin reactions occurring from contact with chemicals can be classified as either irritation or sensitization. Both reactions present as inflammation of the skin; the difference between irritation and sensitization is the mechanism responsible for this inflammation. Primary irritation is direct inflammation in response to injury to the skin produced by the eliciting chemical. Irritation is a locally mediated response ranging from mild reversible inflammation to severe ulceration progressing to necrosis. Sensitization is manifested as indirect inflammation mediated by components of the immune system in response to activation by the eliciting chemical (8). Dermal sensitization is usually a delayed hypersensitivity or cellular immunologic reaction. Although both types of reactions can appear grossly similar in experimental animals and may even be produced by the same agent, it is possible to distinguish between them. Irritation is an immediate response and can be produced upon first contact with the chemical, whereas sensitization requires at least one innocuous "conditioning" exposure before a reaction can be elicited.

Irritative responses usually require a relatively high concentration or dose of the offending chemical, whereas sensitization reactions may occur in response to minute quantities. Essentially all individuals in a population will express an irritative response to a reactive chemical, provided the dose is high enough, whereas only a fraction of the population normally becomes sensitized to the same chemical. A fully developed response can be produced by first contact with an irritant, but initial contact with a sensitizer produces no reaction

(a conditioning exposure is necessary). Unless there is accumulation of damage, subsequent exposures to an irritant produce inflammation of essentially similar intensity/severity, whereas the reaction to a sensitizer often increases over 2 to 4 exposures after the initial contact. An irritant produces inflammation of rapid onset with short duration, whereas a sensitization reaction is somewhat delayed and prolonged. The inflammatory response to an irritant may spread beyond the area of contact, whereas sensitization reactions are usually circumscribed.

The features of irritation and sensitization have been used to establish guidelines for differentiation between the two (5-8). In evaluating a dermal sensitization study it is recommended that the results from a challenge dose in the experimental group (sensitization) be compared with those for the negative control group (irritation) in accordance with the following criteria:

Irritative Responses:

- occur in a large proportion of test animals.
- develop in response to the first or second exposure.
- usually fade within 24 to 48 hours, unless damage is severe.
- may be stronger at challenge to a previously unexposed area of skin (contralateral flank).

Sensitization Reactions:

- occur in only a few animals, unless the compound is a potent sensitizer.
- are absent after the initial (conditioning) exposure, but appear in response to subsequent exposures.
- develop slowly, with the intensity/severity of inflammation often greater at 72 to 96 hours than at 24 to 48 hours.
- increase in intensity/severity from one exposure to the next (at sites previously exposed or unexposed).

Dermal irritancy potential is evaluated by the method of Draize *et al.* (9) in which the chemical is applied once, at high concentration, and the resulting acute inflammatory reaction is graded. Evaluation of sensitizing potential is accomplished by repeated application, at lower nonirritating concentrations, over a few weeks. There is then a latent period, usually two weeks, to allow

the immune system to elaborate and increase its specific response to the chemical. A challenge dose is then given, and the resulting inflammatory response is graded. Analysis of the incidence, severity, and timing of the response to the challenge dose estimates the sensitizing potential of the study compound.

DIGL-RP Solid Propellant

DIGL-RP Solid Propellant was evaluated for its ability to elicit a delayed hypersensitivity or cellular immunologic reaction via contact with the skin. DIGL-RP produced no response indicative of the potential to elicit dermal sensitization when evaluated according to the method of Buehler and Griffith (5-7).

Sensitization produced by DIGL-RP would have been detected by this study. A hypersensitivity-type response was reliably elicited by DNCB in the present group of animals. This response to DNCB was characteristic of that observed previously at the Letterman Army Insititute of Research (10). Although DNCB is capable of producing primary irritation, the characteristics of the responses observed in this study are indicative of a reaction due to sensitization. The concentration of DNCB used for induction and challenge is too low to produce primary irritation. Also, the response to DNCB was observed primarily after two or more exposures.

Because the guinea pig exhibits a somewhat lower sensitizing responsiveness than does man, this result does not guarantee that DIGL-RP will not sensitize humans. However, it does indicate that DIGL-RP Solid Propellant is unlikely to sensitize humans and its potential is low enough to permit its evaluation in man.

CONCLUSION

DIGL-RP Solid Propellant possesses minimal sensitizing potential, as it did not induce a dermal sensitization reaction under conditions of this study.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1983; DTIC No. ADA 127846.

2. Buehler dermal sensitization test. LAIR Standard Operating Procedure OP-STX-82. Presidio of San Francisco, CA: Letterman Army Institute of Research, 18 May 1984.

3. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Dermal sensitization. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.

4. Landsteiner K, Jacobs J. Studies on sensitization of animals with simple chemical compounds. J Exp Med 1935; 61:643-656.

5. Buehler EV. Delayed contact hypersensitivity in the guinea pig. Arch Dermatol 1965; 91:171-175.

6. Griffith JF. Predictive and diagnostic testing for contact sensitization. Toxicol Appl Pharmacol 1969; Suppl 3:90-102.

7. Buehler EV, Griffith JF. Experimental skin sensitization in the guinea pig and man. In: Maibach HI, ed. Animal models in dermatology. Edinburgh: Churchill Livingstone, 1975:56-66.

8. Mathias CGT. Chemical and experimental aspects of cutaneous irritation. In: Marzulli FN, Maibach HF, eds. Dermatotoxicology. Washington, DC: Hemisphere Publishing Corporation, 1983: 167-168.

9. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944; 82:377-390.

10. Hiatt GFS, Morgan EW, Korte DW. Dermal sensitization potential of guanidine hydrochloride in guinea pigs. Toxicology Series 84. Presidio of San Francisco, CA: Letterman Army Institute of Research. Institute Report No. 210, January 1986.

Appendix A: CHEMICAL DATA

Chemical Name: DIGL-RP Solid Propellant

LAIR Code Number: TP57

Physical State: Solid black cylinders (stick configuration)

Preparation of test substance for dosing: The cylinders of DIGL-RP were ground under liquid nitrogen using a Spex freezer mill. After grinding, the powder was sieved through an 80-mesh screen.

Chemical analysis:

DEGDN was the only major component of DIGL which could be easily analyzed. For analysis, samples of DIGL powder were added to individual 100 ml volumetric flasks.[1] After dilution to volume with 90% ethanol, a second 1:100 dilution was performed. These solutions were analyzed by HPLC. Standards consisted of solutions of DEGDN in ethanol, ranging in concentration from 164.5 to 670.5 µg/ml. Analysis of DEGDN by HPLC was performed under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm, Brownlee Labs, Inc., Santa Clara, CA); solvent system, 40% water - 60% acetonitrile); flow rate, 0.9 ml/min; wavelength monitored, 210 nm.[2] Under these conditions, DEGDN eluted with a retention time of approximately 5.4 min. The results from the analysis of standards and DIGL powder samples are presented in Tables 1 and 2.

Table 1. Analysis of Standards

Concentration of Standard (µg/ml)	Peak Area* ($\times 10^{-7}$)
164.5	0.94
191.0	1.09
275.5	1.60
299.4	1.74
362.0	2.08
399.6	2.31
444.4	2.52
539.8	3.07
585.0	3.32
670.5	3.79

*Average of 2 determinations

Equation for line by linear regression analysis:

$Y = 5.62 \times 10^4 X + 3.51 \times 10^5$, $r^2 = 0.9999$

Appendix A (cont.): CHEMICAL DATA

Table 2. Analysis of DIGL Powder

Weight of DIGL Analyzed (mg)	Dilution Factor	Peak Area ($\times 10^{-7}$)	Conc. of DEGDN in DIGL (weight %)*
111.7	100	2.45	38.5
112.6	100	2.46	38.3
100.1	100	2.21	38.7

*Calculated using the equation for the standard curve as follows:
$= \{[\text{Peak Area} - 3.51 \times 10^5]/5.62 \times 10^4\} \div \text{wgt DIGL (mg)} \times 10.$

The average value for the concentration of DEGDN in DIGL was 38.5% and this agrees closely with the value of 36.70 ± 1.50 reported in the manufacturer's data sheet.

Stability:

The aqueous stability of the DEGDN component in the DIGL powder was examined.[3] The amount of DEGDN in aqueous DIGL suspensions was determined immediately after preparation of a suspension and again 24 hrs later. The study was conducted as follows. A suspension of DIGL in 1% gum tragacanth (200 mg/ml) was prepared. Three 1 ml aliquots were removed from the suspension immediately after preparation and again 24 hrs later. The 1 ml samples were transferred to individual 100 ml volumetric flasks. After diluting to volume with ethanol, the flasks were shaken well. A sample from each was analyzed by HPLC as described above. The average of the peak area values was 4.03 ± 0.12 for the 0 time samples and 4.10 ± 0.14 for the 24-hour samples. These results indicate that there was no decomposition of DEGDN in 1% gum tragacanth for a period of 24 hours.

Source: Radford Army Ammunition Plant, Radford, Virginia
(prime contractor: Hercules, Inc., Wilmington, Delaware)

Lot No.: RAD83M001S169

---

1 Wheeler CW. Toxicity Testing of Propellents. Laboratory Notebook #85-12-023, p. 51-61. Letterman Army Institute of Research, Presidio of San Francisco, CA.

2 Wheeler CW. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p. 58. Letterman Army Institute of Research, Presidio of San Francisco, CA.

3 Wheeler CW. Toxicity Testing of Propellents. Laboratory Notebook #85-12-023, p. 24-42. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL ANALYSIS

Manufacturer's Data Sheet for DIGL-RP Formulation

Ingredients	Finished Propellant Percentage
Nitrocellulose (13.05 ±0.05% Nitrogen) (6-12 seconds viscosisty)	62.5 ±2.00
Diethyleneglycol Dinitrate (DEGDN)	36.70 ±1.50
Ethyl Centralite (EC)	0.25 0.25 ±0.05
Akardit II	0.25 0.45 ±0.15
Magnesium Oxide	0.05 Max
Graphite (Chg 5)	0.05 Max
	100.00

Appendix A (cont.): CHEMICAL DATA

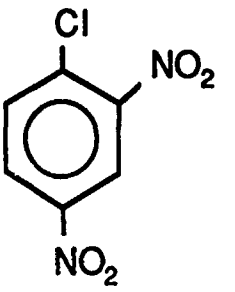
POSITIVE CONTROL

Chemical Name: 1-Chloro-2,4-dinitrobenzene

Alternate Chemical Name: 2,4-Dinitrochlorobenzene

Chemical Abstracts Service Registry Number: 97-00-7

Chemical Structure:



Molecular Formula: $C_6H_3N_2O_4Cl$

Molecular Weight: 202.6

Physical State: Yellow crystals

Melting Point: 52–54° C[1]

Purity: The compound was designated as 95% pure by source.

Analytical Data: Chemical analysis was performed as follows: Infrared spectra were obtained with a Perkin-Elmer 983 spectrometer.[2] Proton magnetic resonance (NMR) spectra were recorded on a Varian XL300 instrument with tetramethylsilane as the internal standard and chemical shifts expressed as parts per million (d).[3] Low resolution GC-MS analysis was performed with a Kratos MS-25RFA (30 m DB-1 capillary column).[4]

---

1 Windholz M, ed. The Merck Index. 10th ed. Rahway, NJ: Merck and Co., Inc., 1983:300.

2 Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, p. 9-10. Letterman Army Institute of Research, Presidio of San Francisco, CA.

3 *Ibid.* p. 11-12.

4 *Ibid.* p. 13-16.

Appendix A (cont.): CHEMICAL DATA

The following data were obtained: IR (KBr): 3443, 3104, 2877, 1963, 1829, 1801, 1756, 1705, 1604, 1591, 1542, 1349, 1246, 1156, 1046, 917, 902, 850, 835, 749, 732 cm^{-1}. The IR spectrum was very close to the Sadtler reference spectrum.[5] Differences were due to the much finer spectral resolution obtained on the P-E 983 instrument. NMR ($CDCl_3$): d 7.78 (1 H, d, J = 8.7 Hz), 8.38 (1 H, q, J_{ortho} = 8.7 Hz, J_{meta} = 3.6 Hz), 8.74 (1 H, d, J_{meta} = 2.4 Hz). The spectrum of DNCB was identical to the Aldrich reference spectrum.[6] GC-MS Analysis: A plot of the total ion current versus scan number showed one major peak for DNCB with only traces of other compounds (not identified). Molecular ion masses (m/z) of 202 and 204 confirmed the identity of the major peak as DNCB.[7]

Lot Number: 11F-0543

Source: Sigma Chemical Co.
St. Louis, MO

---

[5] Sadtler Research Laboratory, Inc., Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared spectrogram #964.

[6] Pouchert CJ. The Aldrich Library of NMR Spectra. Vol. 1, 2nd ed. Milwaukee: Aldrich Chemical Co., 1981:1173, spectrum D.

[7] Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, p. 13-15. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix B: ANIMAL DATA

Species: *Cavia porcellus*

Strain: Hartley, albino

Source: Charles River Breeding Laboratories
Kingston, NY

Sex: Male

Date of Birth: 17 March 1986

Method of randomization: Weight bias, stratified animal allocation

Animals in each group: 15 male animals

Condition of animals at start of study: Normal

Identification procedures: Ear tag.

Pretest conditioning: Quarantine/acclimation 4 - 15 April 1986

Body weight at dosing: 252 - 361 g

Justification: The laboratory guinea pig has proven to be a sensitive and reliable model for detection of delayed hypersensitivity from dermal contact.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

Date	Event
4 Apr 86	Animals arrived at LAIR. Animals were examined, weighed, placed in cages, and fed. Animals were assigned ear tags. Two animals were submitted for necropsy quality control.
4-15 Apr 86	Animals were checked daily.
4,7,14,21,28 Apr, 5,12,16 May 86	Animals were weighed.
14 Apr 86	Animals were randomized into four groups (experimental, positive control, negative control, vehicle control) of 15 animals each.
14,21,28 Apr 86	Study animals, except negative control group, were clipped and shaved.
15,22,29 Apr 86	Study animals, except negative control group, were given induction dose.
16,23,30 Apr 86	Study animals, except negative control group , were scored for 24-hr skin reaction.
17,24 Apr, 1 May 86	Study animals, except negative control group , were scored for 48-hr reaction.
18,25 Apr, 2 May 86	Study animals, except negative control group , were scored for 72-hr reaction.
12 May 86	Study animals were clipped and shaved.
13 May 86	Study animals were given challenge dose.
14 May 86	Study animals were scored for 24-hr reaction.
15 May 86	Study animals were scored for 48-hr reaction.
16 May 86	Study animals were scored for 72-hr reaction. All animals were delivered to Necropsy Suite.

Appendix D: INDIVIDUAL ANIMAL SCORES

GROUP: ONE

COMPOUND: DNCB

ANIMAL NUMBER	FIRST INDUCTION			SECOND INDUCTION			THIRD INDUCTION			CHALLENGE DOSE					
										RIGHT FLANK			LEFT FLANK		
	24 H	48 H	72 H	24 H	48 H	72H	24 H	48 H	72H	24 H	48 H	72 H	24 H	48 H	72 H
86E0131	1	1	1	2	2	2	2	1	2	1	2	1	1	1	2
86E0133	1	1	1	2	1	1	2	1	1	1	2	2	1	2	2
86E0138	0	0	0	2	2	2	1	2	2	2	2	1	2	2	1
86E0140	1	0	0	2	2	2	2	1	2	2	1	1	2	1	1
86E0143	1	1	1	2	2	2	2	1	1	1	2	2	2	2	2
86E0150	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
86E0151	1	1	0	1	1	2	1	1	1	1	1	1	1	2	1
86E0155	0	1	1	1	1	1	1	2	2	1	1	2	1	1	1
86E0165	1	1	1	1	1	1	2	1	2	1	1	1	1	2	2
86E0167	0	0	1	2	2	2	1	1	1	2	2	1	3	3	2
86E0187	1	1	0	1	2	2	2	2	2	3	2	2	3	2	2
86E0191	0	0	0	1	1	1	1	1	1	1	1	1	1	2	2
86E0192	1	1	1	1	1	1	1	1	1	1	2	2	1	2	1
86E0193	0	0	0	2	1	2	2	1	1	0	0	0	2	3	2
86E0195	0	0	0	1	2	1	1	1	1	2	2	2	2	2	2

Appendix D (cont.): INDIVIDUAL ANIMAL SCORES

GROUP: TWO

COMPOUND: ISOTONIC SALINE

ANIMAL NUMBER	FIRST INDUCTION			SECOND INDUCTION			THIRD INDUCTION			CHALLENGE DOSE					
										RIGHT FLANK			LEFT FLANK		
	24 H	48 H	72 H	24 H	48 H	72H	24 H	48 H	72H	24 H	48 H	72 H	24 H	48 H	72 H
86E0145	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0148	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0156	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0159	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0164	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0166	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0168	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0170	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0174	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0177	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0180	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0181	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0182	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0185	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0
86E0186	0	0	0	0	0	0	0	0	0	N/A	N/A	N/A	0	0	0

Appendix D (cont.): INDIVIDUAL ANIMAL SCORES

GROUP: THREE

COMPOUND: DIGL-RP

ANIMAL NUMBER	FIRST INDUCTION			SECOND INDUCTION			THIRD INDUCTION			CHALLENGE DOSE					
										RIGHT FLANK			LEFT FLANK		
	24 H	48 H	72 H	24 H	48 H	72H	24 H	48 H	72H	24 H	48 H	72 H	24 H	48 H	72 H
86E0134	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
86E0135	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0144	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0149	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0152	0	0	1	0	0	0	0	0	0	N/A*					
86E0161	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0162	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
86E0163	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0169	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
86E0171	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0178	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0184	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0188	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0190	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86E0194	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Animal not shaved as scheduled, so removed from study.

Appendix D (cont.): INDIVIDUAL ANIMAL SCORES

GROUP: FOUR

COMPOUND: NEGATIVE CONTROL

ANIMAL NUMBER	FIRST INDUCTION			SECOND INDUCTION			THIRD INDUCTION			CHALLENGE DOSE					
										RIGHT FLANK			LEFT FLANK		
	24 H	48 H	72 H	24 H	48 H	72H	24 H	48 H	72H	24 H	48 H	72 H	24 H	48 H	72 H
86E0136	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0137	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0141	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0142	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0146	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0153	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0154	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0157	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0158	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0160	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0173	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0176	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0179	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0
86E0183	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A†	0	0
86E0189	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	0

† Wrappings were left in place 24 hours, so animal was not scored.

Appendix E: INDIVIDUAL BODY WEIGHTS (grams)

DNCB

Animal Number	DAY OF STUDY							
	0*0	06	013	7	14	21	28	32
86E0131	192	223	275	307	337	373	406	398
86E0133	217	248	310	350	399	451	505	510
86E0138	194	223	266	292	324	348	384	384
86E0140	190	223	263	307	337	386	425	432
86E0143	229	284	361	420	481	558	613	630
86E0150	196	220	260	300	347	371	411	406
86E0151	199	229	290	320	358	400	433	430
86E0155	206	232	280	318	364	414	459	461
86E0165	197	232	285	318	353	396	437	425
86E0167	214	248	284	312	348	381	415	414
86E0187	210	249	302	347	384	422	478	484
86E0191	210	252	296	334	374	429	479	485
86E0192	217	245	287	304	340	380	426	425
86E0193	195	229	269	312	329	367	399	398
86E0195	217	248	282	313	354	395	435	441
MEAN	205.5	239.0	287.3	323.6	361.9	404.7	447.0	448.2
Standard Deviation	11.7	16.9	24.8	31.2	38.7	50.1	56.5	61.8
Standard Error	3.0	4.4	6.4	8.0	10.0	12.9	14.6	16.0

* Q represents quarantine period.

Appendix E (cont.): INDIVIDUAL BODY WEIGHTS (grams)

ISOTONIC SALINE

Animal Number	DAY OF STUDY							
	Q*0	06	013	7	14	21	28	32
86E0145	217	258	313	356	391	446	501	519
86E0148	227	253	302	340	373	398	440	444
86E0156	187	218	274	328	368	412	466	471
86E0159	212	242	290	322	362	392	439	445
86E0164	194	228	275	332	377	415	461	470
86E0166	184	210	255	292	320	360	397	404
86E0168	226	263	282	324	362	405	454	450
86E0170	221	276	333	376	431	474	533	537
86E0174	199	240	295	338	375	427	460	468
86E0177	197	226	284	337	366	414	458	483
86E0180	197	222	265	315	356	403	457	460
86E0181	203	230	260	288	319	338	370	366
86E0182	189	236	279	335	380	420	469	479
86E0185	192	226	290	331	371	405	445	453
86E0186	209	243	283	321	346	391	423	426
MEAN	203.6	238.1	285.3	329.0	366.5	406.7	451.5	458.3
Standard Deviation	14.2	18.2	20.2	21.8	27.0	31.9	38.4	41.7
Standard Error	3.7	4.7	5.2	5.6	7.0	8.2	9.9	10.8

* Q represents quarantine period.

Appendix E (cont.): INDIVIDUAL BODY WEIGHTS (grams)

DIGL-RP SOLID PROPELLANT

Animal Number	DAY OF STUDY							
	0*0	06	013	7	14	21	28	32
86E0134	203	234	264	300	335	375	397	405
86E0135	188	218	274	299	349	380	437	440
86E0144	192	230	282	324	369	415	471	478
86E0149	228	270	318	357	398	417	467	475
86E0152	195	229	274	312	348	398	480	459
86E0161	203	230	280	326	373	410	462	471
86E0162	205	224	267	327	386	441	491	497
86L0163	180	212	252	301	338	376	420	426
86L0169	210	256	298	340	386	440	486	488
86E0171	204	233	292	337	388	425	473	484
86E0178	220	254	305	343	378	411	460	468
86E0184	182	221	286	332	374	429	490	495
86E0188	212	236	286	335	384	436	490	479
86E0190	235	272	301	334	370	401	441	445
86E0194	211	247	280	320	341	362	392	400
MEAN	204.5	237.7	283.9	325.8	367.8	407.7	457.1	460.7
Standard Deviation	15.8	18.2	17.1	16.9	20.4	25.3	32.9	31.0
Standard Error	4.1	4.7	4.4	4.4	5.3	6.5	8.5	8.0

* Q represents quarantine period.

Appendix E (cont.): INDIVIDUAL BODY WEIGHTS (grams)

Negative Control

Animal Number	DAY OF STUDY							
	0*0	06	013	7	14	21	28	32
86E0136	185	217	254	301	334	369	409	410
86E0137	195	226	282	330	369	399	437	440
86E0141	207	252	297	351	393	445	484	485
86E0142	230	265	305	363	413	468	512	520
86E0146	190	227	282	332	375	440	486	487
86E0153	222	254	305	333	389	428	474	469
86E0154	206	245	289	335	386	433	478	477
86E0157	188	227	280	327	381	440	486	495
86E0158	211	262	321	380	432	486	542	546
86E0160	208	232	279	326	375	416	469	461
86E0173	193	227	267	318	364	409	451	469
86E0176	178	217	260	319	363	410	467	463
86E0179	211	238	285	338	388	422	479	478
86E0183	200	243	291	341	379	418	454	437
86E0189	183	218	266	319	354	404	449	452
MEAN	200.5	236.7	284.2	334.2	379.7	425.8	471.8	472.6
Standard Deviation	14.9	16.1	18.2	19.3	23.4	28.5	31.2	33.2
Standard Error	3.8	4.2	4.7	5.0	6.0	7.4	8.1	8.6

* Q represents quarantine period.

Appendix F: PATHOLOGY REPORT

GLP Study # 85026

Principal Investigator: Y. Johnson APC# LLE0

I. INTRODUCTION

Study: Buehler Dermal Sensitization
Animal: Guinea Pig (Hartly-Albino)/2 months/male
Reference: SOP-OP-STX-84

II. SUMMARY OF PROCEDURES

Euthanasia: Sodium Pentobarbital.
Fixative: 10% buffered formalin.
Histopathology: None
Clinical Lab: None

III. GROSS FINDINGS

DOSE GROUP 1 - POSITIVE CONTROL (DNCB)
(All live animals)

LAIR ACC#	ANIMAL ID#	OBSERVATION
39559	86D00131	Not remarkable (NR)
39560	86D00133	Liver - multiple white foci, minimal.
39565	86D00138	NR
39566	86D00140	Liver - multiple white foci, mild.
39569	86D00143	Liver - multiple white foci, minimal.
39575	86D00150	Liver - multiple white foci, minimal.
39576	86D00151	NR
39580	86D00155	Liver - multiple white foci, minimal.
39590	86D00165	Liver - multiple white foci, minimal.
39592	86D00167	Liver - multiple white foci, moderate.
39610	86D00187	Liver - multiple white foci, minimal.
39614	86D00191	NR
39615	86D00192	Liver - multiple white foci, minimal.
39616	86D00193	Liver - multiple white foci, mild.
39618	86D00195	NR

Appendix F (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 85026

DOSE GROUP 2 - SALINE CONTROL
(All live animals)

LAIR ACC#	ANIMAL ID#	OBSERVATION
39571	86D00145	Liver - Multiple white foci, minimal.
39573	86D00148	Liver - multiple white foci, minimal.
39581	86D00156	Liver - multiple white foci, minimal.
39584	86D00159	Liver - multiple white foci, minimal.
39589	86D00164	NR
39591	86D00166	Liver - multiple white foci, minimal.
39593	86D00168	NR
39595	86D00170	Liver - multiple white foci, minimal.
39598	86D00174	Liver - multiple white foci, minimal.
39600	86D00177	Liver - multiple white foci, minimal.
39603	86D00180	Liver - multiple white foci, minimal.
39604	86D00181	Liver - multiple white foci, minimal.
39605	86D00182	NR
39608	86D00185	NR
30609	86D00186	Liver - multiple white foci, minimal.

DOSE GROUP 3 - DIGL-RP
(All live animals)

LAIR ACC#	ANIMAL ID#	OBSERVATION
39561	86D00134	NR
39562	86D00135	NR
39570	86D00144	Liver - multiple white foci, minimal.
39574	86D00149	NR
39577	86D00152	NR
39586	86D00161	NR
39587	86D00162	NR
39588	86D00163	Liver - multiple white foci, minimal.

Appendix F (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 85026

DOSE GROUP 3 (Continued)

39594	86D00169	NR
39596	86D00171	NR
39601	86D00178	NR
39607	86D00184	NR
39611	86D00188	Liver - minimal white foci.
39613	86D00190	Liver - multiple white foci, minimal.
39617	86D00194	NR

DOSE GROUP 4 - NEGATIVE CONTROL
(All live animals)

LAIR ACC#	ANIMAL ID#	OBSERVATION
39563	86D00136	NR
39564	86D00137	NR
39567	86D00141	Liver - multiple white foci, minimal.
39568	86D00142	NR
39572	86D00146	Liver - multiple white foci, minimal.
39578	86D00153	Liver - multiple white foci, minimal.
39579	86D00154	NR
39582	86D00157	Liver - multiple white foci, mild.
39583	86D00158	NR
39585	86D00160	Liver - multiple white foci, minimal.
39597	86D00173	Liver - multiple white foci, minimal.
39599	86D00176	NR
39602	86D00179	NR
39606	86D00183	Liver - multiple white foci, minimal.
39612	86D00189	NR

Appendix F (cont.): PATHOLOGY REPORT

Pathology Report
GLP Study 85026

IV. SUMMARY COMMENTS: Lobular liver necrosis in guinea pigs is not an uncommc finding in normal animals and, as yet, has an unexplained etiology. No significant gross lesions were present in any of the animals included in this study.

MICHAEL V. SLAYTER, DVM
MAJ, VC
C, Comparative Pathology Branch

5 June 1986

Distribution List

Commander
US Army Biomedical Research and Development Laboratory (12)
ATTN: SGRD-UBZ-C
Fort Detrick, Frederick, MD 21701-5010

Defense Technical Information Center (DTIC) (2)
ATTN: DTIC-DLA
Cameron Station
Alexandria, VA 22304-6145

US Army Medical Research and Development Command (2)
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21701-5012

Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

Chief
USAEHA Regional Division, West
Fitzsimmons AMC
Aurora, CO 80045

Chief
USAEHA Regional Division, North
Fort George G Meade, MD 20755

Chief
USAEHA Regional Division, South
Bldg. 180
Fort McPherson, GA 30330

Commander
USA Health Services Command
ATTN: HSPA-P
Fort Sam Houston, TX 78234

Commander US Army Materiel Command
ATTN: AMSCG
5001 Eisenhower Avenue
Alexandria, VA 22333

Commander
US Army Environmental Hygiene Agency
ATTN: Librarian, HSDH-AD-L
Aberdeen Proving Ground, MD 21010

Dean
School of Medicine
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014

Commander
US Army Materiel Command
ATTN: AMCEN-A
5001 Eisenhower Avenue
Alexandria, VA 22333

HQDA
ATTN: DASG-PSP-E
Falls Church, VA 22041-3258

HQDA
ATTN: DAEN-RDM
20 Massachusetts, NW
Washington, D.C. 20314

CDR, US Army Toxic and Hazardous Material Agency
ATTN: DRXTH/ES
Aberdeen Proving Ground, MD 21010

Commandant
Academy of Health Sciences
United States Army
ATTN: Chief, Environmental Quality Branch
Preventive Medicine Division (HSHA-IPM)
Fort Sam Houston, TX 78234